View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Biochimica et Biophysica Acta 1792 (2009) 625–633

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Zeroing in on LRRK2-linked pathogenic mechanisms in Parkinson's disease

Saskia Biskup [a,b], Andrew B. West [c,*]

[a] Institute of Medical Genetics, University of Tübingen, Tübingen, Germany
[b] Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany
[c] Center for Neurodegeneration and Experimental Therapeutics, Department of Neurology, University of Alabama School of Medicine, Civitan International Research Center, Suite 516, 1719 6th Avenue South, Birmingham, AL 35294, USA

ARTICLE INFO

Article history:
Received 7 August 2008
Received in revised form 9 September 2008
Accepted 29 September 2008
Available online 10 October 2008

Keywords:
Leucine-rich repeat kinase 2
Dardarin
Neurodegeneration
Kinase
Movement disorder
Molecular genetic

ABSTRACT

The frequency and potency of mutations in the LRRK2 gene redefine the role of genetic susceptibility in Parkinson's disease. Dominant missense mutations that fulfill initial criteria for potential gain of function mechanisms coupled with enzymatic activity likely amenable to small molecule inhibition position LRRK2 as a promising therapeutic target. Herein, key observations from the clinic to the test tube are highlighted together with points of contention and outstanding critical issues. Resolution of the critical issues will expedite the development of therapies that exploit LRRK2 activity for neuroprotection strategies.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Parkinson's disease (PD) encompasses a myriad of symptoms traditionally defined by prominent movement related dysfunction initially alleviated through dopamine modification therapies, in addition to non-motor symptoms unresponsive to L-dopa treatment [1]. Recent advances in a pathological understanding of the disease confirm the association of a massive loss of dopaminergic neuromelanin-positive neurons in the substantia nigra pars compacta with the movement disorder phenotype, in conjunction with α-synuclein positive lesions throughout many regions of the brain before and after degeneration in the midbrain [2]. As the most common movement disorder, PD is a major cause of morbidity and mortality and therapies that modify the progressive nature of the disease remain unidentified. Molecular genetic studies in the last decade provide clues to the underlying pathogenic mechanisms and potential targets for therapeutic intervention.

The identification of autosomal-dominant mutations in the *leucine-rich repeat kinase 2* gene (*LRRK2*) in a significant proportion of cases in some populations redefine the role of genetic susceptibility in PD. As opposed to mutations in the α-*synuclein* gene (*PARK1* and *PARK4*) rarely found in PD cases and *parkin* (*PARK2*) mutations common in early onset PD, *LRRK2* (*PARK8*)-linked PD cases more closely associate with the expected phenotype of typical late-onset PD. Moreover, genetic alterations, namely missense mutations, within *LRRK2* that concede to minimum reasonable criteria for disease causality suggest gain-of-function mechanisms potentially amenable to therapeutic intervention. As a protein kinase, LRRK2 belongs to a class of proteins successfully targeted by small molecule inhibitors in the treatment of human disease. Coupling gain-of-function disease associated activity with a protein theoretically modifiable by small molecules, LRRK2 represents a strong target for the development of therapies that slow or halt disease progress. This review dedicates towards highlighting key observations related to LRRK2, questions that need resolution, and suggestions for moving forward.

2. Genetic aspects of *LRRK2* and PD

The *PARK8* locus was initially described in a large Japanese kindred [3]. Soon after, association of the locus on chromosome 12 was replicated in two large and eight smaller additional kindreds, suggesting that association of the *PARK8* locus with PD spans ethnicities and might be a more common cause of PD than previously characterized *PARK* loci [4]. The refined *PARK8* locus includes 29 annotated and putative genes on chromosome 12 comprehended of 13Mbp. To identify the disease-associated gene within *PARK8*, the region was divided to conquer; the smallest genes sequenced first and corresponding mRNA transcripts analyzed for expression. After several misleading sidetracks typical for positional cloning projects, the last of the annotated genes waited on the list, a gene that only had an anonymous and hostile index consisting of more than 30 incompletely annotated exons. An amino acid exchange in the predicted exon 31 was followed by the discovery of another non-

* Corresponding author. Tel.: +1 205 996 6329; fax: +1 205 996 6580.
E-mail address: abwest@uab.edu (A.B. West).

0925-4439/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2008.09.015

synonymous alteration in a highly conserved codon in the 35th predicted exon. Since these variants segregated with disease in the families and initial sequencing of two-thousand control chromosomes failed to identify the variants, the alterations themselves were likely the disease causing mutations underlying *PARK8*. Thus, a common world-spanning effort that took several years of dedicated work resulted in the unambiguous identification of the gene in *PARK8* responsible for PD [4,5].

Finding an appropriate name for the corresponding *PARK8* gene was the first step. Upon searching the databases it became clear that the unruly gene containing the PD-linked mutations belongs to the ROCO family of proteins, an *in silico* predicted group of molecules defined by the Ras of Complex (ROC) GTPase-like domain adjacent to a C-terminal of ROC (COR) linker [6]. Additionally, a kinase domain with similar amino acid composition to MAPKKK (mitogen activated protein kinase kinase kinase) is often present in ROCO proteins together with other regulatory and protein-interaction domains. Unique to the *PARK8* gene is sequence encoding an almost one-thousand amino acid long N-terminus with little homology to any other mammalian protein. Humans have two ROCO family proteins and since human ROCO1 was previously annotated with little consideration towards functionality as leucine-rich repeat kinase 1, the PD-linked gene in *PARK8* had no chance besides *leucine-rich repeat kinase 2* (*LRRK2*, pronounced lurk-two or lark-two). Besides the leucine-rich repeat domain in LRRK2, other potential interaction domains include an WD-40 like domain near the C-terminus and N-terminal repeat structures and some Ankyrin-repeat like sequences, in addition to a Rab-like GTPase domain, all of which arbitrarily neglected from the *LRRK2* gene nomenclature. In addition, the implication of LRRK2 as a kinase despite functional validation could have made the initial LRRK2 designation both inaccurate and misleading, since conserved kinase domains in proteins that are not authentic kinases have been described [7].

At the same time as the R1441C (Exon 31) and the Y1699C (Exon 35) mutation in *LRRK2* were linked with PD in one family from Western Nebraska with probable English origin and in one family from Canada, US, France and Germany with German origin [4], Paisan-Ruiz et al. [5] reported R1441G in four Basque families and Y1699C in one English family and dubbed the protein product of *LRRK2* dardarin after the Basque word dardara, meaning tremor. DiFonzo, Gilks and Nichols with coworkers went on to describe the G2019S mutation [8–10], recognized as the most frequently found *LRRK2* mutation that spans diverse ethnicities. Finally, the *LRRK2* gene in the Japanese kindred responsible for the original identification of the *PARK8* locus was sequenced and the I2020T mutation identified [11]. All four sites (R1441, Y1699, G2019 and I2020) are conserved between mouse and human *LRRK2*; all are segregating in families with disease and all are therefore causative for disease within reasonable doubt. Since the identification of these mutations, additional rare variants potentially linked with PD have been identified (Table 1). Usually the amino acid sequence comprising the PD-linked mutation is present only in mammals or other high-order vertebrates (annotated as 'Conservation medium' in Table 1). For those variants with medium conservation, segregation in families was usually not reported, and for the majority of *LRRK2* variants functional data on kinase or GTPase activity has not been described. Thus, future studies that ideally involve complete sequencing of the *LRRK2* gene in both PD-patients and controls in addition to the incorporation of standardized functional biochemical studies will be needed to determine the potential pathogenicity of the multitude of *LRRK2* non-synonymous alterations.

As the most common *LRRK2* mutation (Table 2), the frequency of G2019S has been described in nearly all well characterized PD case/control cohorts. The mutation is found with frequencies in PD patients ranging from 40% to 0%. Interestingly, some populations display higher proportions of cases with the common G2019S mutation in sporadic cases or patients that report no PD in their immediate family than those with a family background of PD. The immediate assumption of *de novo* G2019S mutation is not supported by a number of studies that demonstrate the ancient ancestry of the G2019S linked haplotype [12–16]. The unprecedented frequency of this relatively penetrant mutation linked with the common neurodegenerative disorder PD perhaps reveals the inherent inaccuracy of classifying PD patients as sporadic or non-familial and may highlight a general underestimation of the role of genetic susceptibility in PD. Beyond the biological implications of the role of LRRK2 in PD, the frequency of known pathogenic mutations in PD has provided valuable insight and will likely continue to clarify the disease in future studies. As already

Table 1
Variations within LRRK2

Variant	Region Within LRRK2	Evidence for Pathogenicity	Kinase activity	GTPase activity	Ref
			In vitro	In vitro	
E10K	N-Term	Conservation medium	n.d.	n.d.	[74]
A211V	N-Term	Conservation medium	n.d.	n.d.	[75]
E334K	N-Term	Conservation medium	n.d.	n.d.	[74]
K544V	N-Term	Conservation medium	n.d.	n.d.	[75]
M712V	N-Term	Conservation medium	n.d.	n.d.	[76]
R793M	N-Term	Conservation medium	n.d.	n.d.	[77]
Q930R	N-Term	Conservation medium	n.d.	n.d.	[77]
S973N	N-Term	Conservation medium	n.d.	n.d.	[78]
R1067Q	LRR	Conservation medium	n.d.	n.d.	[79]
S1096C	LRR	Conservation medium	n.d.	n.d.	[77]
Q1111H	LRR	Conservation medium	n.d.	n.d.	[74]
L1114L	LRR	Segregating	n.d.	n.d.	[4]
L1122V	LRR	No conservation	increased	n.d.	[4,41]
A1151T	LRR	Conservation medium	n.d.	n.d.	[80]
L1165P	LRR	Conservation medium	n.d.	n.d.	[81]
I1192V	LRR	Conservation medium	n.d.	n.d.	[74]
S1228T	LRR	Conservation medium	n.d.	n.d.	[77]
R1325Q	GTPase	Conservation medium	n.d.	n.d.	[82]
I1371V	GTPase	Conservation medium	No change	n.d.	[41,83]
R1441C/G/H	**GTPase**	**Conservation high Segregating**	**increased/ unaltered**	**decreased**	[4,5,22, 46,48,84]
K1468E	GTPase	Conservation medium	n.d.	n.d.	[82]
R1483Q	GTPase	Conservation medium	n.d.	n.d.	[82]
R1514Q	COR	Conservation medium	increased	n.d.	[41,85]
P1542S	COR	Conservation medium	n.d.	n.d.	[85]
V1613A	COR	Conservation medium	n.d.	n.d.	[86]
R1628P	COR	Conservation medium	n.d.	n.d.	[85]
Y1699C	**COR**	**Conservation high Segregating**	**increased/ unaltered**	**n.d.**	[4,5,41, 44,46]
R1728L,H	COR	Conservation medium	n.d.	n.d.	[76]
L1795F	COR	Conservation high	n.d.	n.d.	[74]
M1869T/V	Kinase	Conservation medium	n.d.	n.d.	[8,85]
1874Stop	Kinase	Conservation medium	n.d.	n.d.	[8]
R1941H	Kinase	Conservation medium	decreased/ unaltered	n.d.	[44,46,87]
Y2006H	Kinase	Conservation medium	n.d.	n.d.	[88]
I2012T	Kinase	Conservation medium	decreased	n.d.	[4,41, 44,46]
G2019S	**Kinase**	**Conservation high Segregating Drosophila model Mouse model**	**increased**	**n.d.**	[8–10,41, 59,70]
I2020T	**Kinase**	**Conservation high Segregating**	**increased/ decreased/ unaltered**	**n.d.**	[4,41, 44–46]
T2031S	Kinase	Conservation medium	n.d.	n.d.	[88]
N2081D	Kinase	Conservation medium	n.d.	n.d.	[82]
T2141M	C-Term	No conservation	n.d.	n.d.	[76]
R2143H	C-Term	No conservation	n.d.	n.d.	[76]
Y2189C	C-Term	Conservation medium	n.d.	n.d.	[82]
T2356I	C-Term	No conservation	unaltered	n.d.	[46,87]
G2385R	C-Term	Conservation medium	unaltered/ decreased	n.d.	[41,46,85]
V2390M	C-Term	Conservation medium	n.d.	n.d.	[89]
M2397T	C-Term	Conservation medium	n.d.	n.d.	[85]
L2466H	C-Term	Conservation medium	n.d.	n.d.	[76]

Known pathogenic variations are shown in bold. Known polymorphisms that are frequently found in controls are not included. (n.d. not determined).

Table 2
Frequency of LRRK2 mutations

Origin	Variant tested (predominant)	sp = sporadic fam = familial	Reference
North Africa	G2019S	sp 41% fam 37%	[15,17]
Arabs	G2019S	sp 39% fam 36%	
Ashkenazim	G2019S	sp 13% fam 30%	[17,90]
	G2019S	sp 10% fam 28%	
Germany	Complete Seq		[77,91]
	(R793M)	sp 2% fam 15%	
	G2019S	sp + fam 0,5%	
Portugal	G2019S	sp 4% fam 14%	[17,92]
		sp + fam 6%	
Italy	G2019S (6,6%)	fam 10%	[17,93]
	R1441C (4,3%)	sp 2% fam 4%	
Spain	R1441G/G2019S	fam 5%, sp + fam 8%	[17,94]
	G2019S	sp 3% fam 4%	
United States	G2019S	1–2%	[18]
	G2019S	fam 6–7%	
Russia	G2019S	sp 0,5% fam 5,9%	[17,86]
	G2019S	sp 1% fam 0%	
Belgium	R1441C	sp + fam 3,29%	[82]
Chile	G2019S	sp 3% fam 3%	[17]
Ireland	G2019S	sp<1% fam 3%	[17]
France	G2019S	sp + fam 2,9%	[95]
Australia	G2019S	sp + fam 1,5%	[96]
Brazil	G2019S	sp + fam 2%	[97]
Norway	G2019S	sp + fam 2%	[17,98]
	G2019S	sp 1% fam 1%	
UK	G2019S	fam 1,8%	[9,17]
	G2019S	sp 1% fam 2%	
Sweden	G2019S	sp 2% fam 0%	[17]
Japan	G2019S	sp<1% fam 2%	[17]
India	G2019S	sp<1% fam 0%	[17]
Canada	G2019S	sp + fam 0%	[99,100]
Greece	G2019S	sp + fam 0%	[101]
Poland	G2019S	sp + fam 0%	[102]
China	G2385R, risk factor	sp + fam 9%	[103,104]
	P1628R, risk factor	sp + fam 3%	

alluded to, the true frequency of *LRRK2* mutations in PD will wait on the successful discernment between benign rare variants and pathogenic mutations.

3. Clinical and pathological aspects of LRRK2 associated disease

The clinical signs of patients with *LRRK2* mutations are perhaps surprisingly uniform for movement disorder specialists in frequent contact with cases caused by mutation in a known *PARK* locus. Large proportions of *LRRK2*-patients become symptomatic and seek the treatment of neurologists upon the onset of resting tremor and bradykinesia, similar to sporadic cases. Age at onset can vary from 30 to 90 years with a mean age of onset near 60 years, although clinic and referral bias may be responsible for an artificially lower age of onset for *LRRK2*-mutation carriers. The *LRRK2*-associated clinical phenotype can endure a few years to decades with no large difference to sporadic *LRRK2*-negative cases when adjusted for age and sex. Statistically significant differences between symptomatic *LRRK2*-mutation carriers and sporadic patients are therefore hard to determine. Since G2019S carriers are by far the most abundant known *LRRK2*-linked cases, the effects of this mutation are well described and tremor seems to be more abundant with a slightly more benign disease course compared to sporadic *LRRK2*-negative cases [17], with the usual caveats of clinic-bias in mind. Further, G2019S patients have a lower risk of developing cognitive impairment and hyposmia, needed dopamine-replacement therapy later and presented with less frequent drug-induced dyskinesias [17]. An important aspect is the penetrance of disease in a particular mutation background since G2019S carriers have been identified in apparently neurologically normal individuals including subjects over eighty years of age [18]. Percentages of penetrance are variable between studies with around 30% for subjects in their fifties to up to 80% in subjects older than eighty years of age [12,13,17,19]. Larger population-based studies in the future will help define the true penetrance of *LRRK2* mutations in different ethnicities and therefore aide genetic counseling, particularly if a *LRRK2*-specific disease therapy becomes available. Nevertheless, in relative comparison, pathogenic-proven *LRRK2* mutations are highly penetrant.

As rationally-designed therapeutic strategies become implemented, an important challenge to the research community lies in developing tools for early diagnosis. Although the true risk factors are not yet known, sequencing of patients and controls necessarily leads to the identification of presymptomatic family members and carriers of mutations and risk factors. A number of imaging tools including ultrasound, MRI and PET scans are available mostly on a research basis to uncover early cell loss, amyloid positive aggregates, changes in dopamine metabolism, and neuronal inflammation. Studies suggest that the neurochemical changes that accompany *LRRK2*-associated PD cannot be distinguished from idiopathic PD [20]. Since the field of biomarkers in PD is yet in its infancy, one potential approach might utilize LRRK2 function in the periphery to assess the progression of PD. The possible presence of LRRK2 protein in leukocytes or lymphoblastoid cells [21], if confirmed with validated antibodies, may represent a viable approach.

When *LRRK2*-mutations have taken their course and neuropathologists have the opportunity for analysis, the majority of brains now described in the literature suggest *LRRK2* mutations cause standard Lewy body pathology typical for idiopathic cases (summarized in Table 3), despite initial indications that *LRRK2* mutations cause highly variable pathology. Pathology outside of the expected phenotype for PD includes four affected members of a family from Western-Nebraska with the first described R1441C mutation [4]. One case demonstrates typical brain stem pathology, one with diffuse Lewy body pathology and the other two with either tauopathy or pure nigral degeneration without Lewy body formation. In addition, two subjects from the German-Canadian family with the first Y1699C mutation showed degeneration of the substantia nigra, Lewy bodies and neurites were seen in the limbic gray matter in one patient, the other had additional degeneration of the anterior horn suggesting the coexistence of amyloid lateral sclerosis [4]. Four patients from the Japanese kindred with the I2020T mutation presented with pure nigral degeneration [11]. Of note, the R1441C, the Y1699C and the I2020T are rare mutations and can cause both clinical and pathological phenotype well outside of the accepted PD spectrum. In contrast, the majority of sporadic and familial G2019S patients with a diagnosis of PD demonstrate substantia nigra degeneration and Lewy body pathology (Table 3). As there is variation in the combination of clinical symptoms in PD there is clear variation in end stage pathology, particularly in

Table 3
Pathology of LRRK2 mutation carriers with a clinical diagnosis of typical PD

Origin	Mutation	Type	Pathology	Cases	Ref
Spain	G2019S	Sporadic	LB, SNpc degeneration	1	[105]
	G2019S	Sporadic	No inclusions, SNpc degeneration	1	[105]
Spain	R1441R	Sporadic	LB, SNpc degeneration	1	[105]
UK	G2019S	Sporadic	LB, SNpc degeneration	3	[9]
USA	G2019S	Sporadic	LB, SNpc degeneration	6	[106,107]
			No inclusions, SNpc degeneration	1	
USA	G2019S	Familial	LB, SNpc degeneration	4	[106–108]
			Tauopathy, SNpc degeneration	1	
USA	R1441C	Familial	LBs, SNpc degeneration	2	[4,109]
			Ub-only inclusions	1	
German-Canadian	Y1699C	Familial	Ub-only inclusions and SNpc degeneration	2	[4,87,109]
UK			LB, SNpc degeneration	1	
Japan	I2020T	Familial	No inclusions, SNpc degeneration	4	[11]
Italy	I1371V	Familial	LB, SNpc degeneration	1	[110]

families where LRRK2 mutations can be detected in cases that otherwise might not fulfill clinical criteria for typical late-onset PD. Rather than installing LRRK2 upstream of commonly considered pathways in rare cases, it is more convincing to associate LRRK2 mutations with the most frequently found type of pathology, namely the accumulation of proteins into Lewy bodies in refined brain regions with dopaminergic neuronal cell loss.

4. LRRK2 expression and localization

The LRRK2 protein consists of 2527 amino acids translated from 51 exons. Initial efforts to identify splicing events or alternative exons in mRNA derived from human brain failed to reveal common alternative *LRRK2* isoforms, although a cryptic splice site in between exons 50 and 51 results in the exclusion of 6 amino acids in a significant proportion of *LRRK2* transcript [22]. All bioinformatically available data about primary, secondary and higher order structure in conjunction with the evolutionary tree of related proteins suggest LRRK2 as a multidomain GTPase and kinase enzyme. LRRK1 and LRRK2 may be the only proteins in the mammalian proteome that combine a functional GTPase with kinase activity into one molecule. In view of upcoming *LRRK2* deficient and over-expressing mouse models, *LRRK1* might be considered for potential functional overlap and compensation effects. Several groups have focused on *LRRK* family expression patterns during development and adulthood, in different cell types and organisms. An idea of *LRRK1* and *LRRK2* localization patterns has recently emerged.

First, the presence of *LRRK2* mRNA was demonstrated through Northern Blot analysis and RT-PCR in adult human brain [4,5]. *In situ* hybridization followed and gave impressions about *LRRK2* distribution throughout the brain of rodents and later distribution in humans. *LRRK2*, although expressed at nominal levels compared to even rare control transcripts, can be detected in several brain regions, primarily striatum, cortex and hippocampus [23–28]. For *LRRK1*, the smaller ROCO-family member with 33 exons and 2038 amino acids, mRNA expression is found in all areas of adult rodent brain by RT-PCR but only very low levels can be detected by *in situ* hybridization [29,30]. In contrast to *LRRK2*, *LRRK1* is more abundant in the developing rodent brain [30]. LRRK1 antibodies are commercially available but specificity and final characterization in brain tissue awaits LRRK1 deficient mouse models. LRRK2 antibodies, available from every major commercial antibody supplier, have flooded the market with reagents with questionable specificity to LRRK2 protein, particularly mouse LRRK2. However, LRRK2 protein is consistently detected in neurons within the cortex, striatum, hippocampus, cerebellum, and in dopaminergic neurons of the substantia nigra [31–33].

Initial experiments with a rabbit polyclonal antibody that detects both mouse and human LRRK2 protein and validated through analysis of LRRK2-null tissue presented a cytoplasmic punctate pattern for LRRK2 protein in neurons on a light microscopy level [33]. Multiple cell fractionation experiments demonstrated that a significant proportion of LRRK2 protein is membrane associated and indeed the protein shows partial co-localization with different markers like lyso- and mitotracker [33]. Electron microscopy further describes LRRK2 co-localization with transport vesicles from the Golgi to the periphery, with endosomes, synaptic vesicles and the cytoskeleton, with localization always remaining on the cytosolic side [33]. The distribution of LRRK2 protein in PD brains, especially localization to Lewy body structures, varies between studies (0 to 100%) depending on the antibody [24,34–38]. The role of LRRK2 in Lewy body formation is unknown. Although Lewy bodies are frequently found in LRRK2-associated disease, LRRK2 itself does not seem to be a major component.

From all tissues examined by RT-PCR, *LRRK2* reaches its highest levels in embryonic and adult kidney [39]. *In situ* hybridization demonstrates a differential expression pattern between *LRRK1* and *LRRK2* in kidneys, as *LRRK2* is predominantly in tubules of the deep renal cortex and *LRRK1* resides mostly in the renal medulla [30]. *LRRK1* and *LRRK2* mRNA demonstrate striking overlap in expression in several other organs, with moderate levels in developing and adult rodent lung, heart and spleen [39]. In summary, LRRK2, as with other PD-linked genes, is widely expressed throughout the body and in cell types and brain regions relevant to PD. From expression patterns, LRRK1 may play a larger role in early development whereas LRRK2 levels significantly increase after birth.

5. LRRK2 protein function: linking PD mutations with GTPase and kinase activity

LRRK2 is one of the largest kinases in the human kinome, with less than ten kinases (mostly predicted kinases) derived from the PIKK family, the Trio family and the MLCK family encoding proteins larger than LRRK2, the majority of which remain uncharacterized on a molecular level. While all large kinase proteins display unique configurations of conserved protein domains, LRRK2 and the related LRRK1 gene encode the only proteins within the mammalian genome that include both kinase and GTPase domains, with the possible exception of the calcium-calmodulin-dependent death-associated protein kinase 1 (DAPK1) that contains a possible GTPase-like domain [6]. Since the most common mutations linking LRRK2 with susceptibility to PD occur within the LRRK2 kinase and GTPase domains, attention and intense speculation immediately revolved around the enzymatic output of LRRK2 protein prior to a functional description of the protein.

As a highly evolutionarily conserved kinase-protein arguably present in even some single-celled organisms with broad expression throughout mammalian development, LRRK2 protein plausibly performs dozens of functions in dozens of cells types. Perhaps the best way to study LRRK2 as a cause and potential therapeutic target for PD revolves around understanding how PD-associated mutant LRRK2 protein deviates from normal protein. The first functional description of LRRK2 protein included a comparison of the most common mutation found in PD cases, G2019S, which localizes to the activation loop kinase sub-domain, together with normal LRRK2 protein sequence as found in unaffected control brain tissue [22]. While the basic biochemical properties of wild-type and mutant protein such as protein localization and stability are comparable, at least in transiently transfected cell lines, the influence of the PD-linked mutations is clear in experiments designed to assess enzymatic activity. The G2019S mutation enhances kinase activity in autophosphorylation assays and phosphorylation of a generic kinase substrate myelin basic protein [22]. These findings, derived from *in vitro* based assays, complement genetic studies that suggest dominant inheritance and likely gain of function pathogenic mechanisms.

LRRK2 belongs to the tyrosine kinase-like protein family in humans [40]. Despite the nomenclature, all described kinase proteins within the tyrosine kinase-like family display serine and/or threonine activity without examples of tyrosine kinase activity. Separation of internal LRRK2 residues labeled during autophosphorylation reveal both serine and threonine kinase activity, typical for tyrosine kinase like proteins [41]. Although the LRRK2 kinase domain displays highest sequence homology to the mixed-lineage kinase (MLK) subfamily of MAPKKK protein kinases, so named due to kinase sub-domain structure resembling both tyrosine and serine/threonine kinases, LRRK2 differs from the consensus critical amino acids that define mixed-lineage kinases [41]. Thus, LRRK1 and LRRK2 protein kinase domains have unique sub-domain characteristics that clearly delineate from MLKs and other MAPKKK proteins. However, like MLKs, LRRK2 forms protein dimers in cells and dimerization is dependent on kinase activity [42]. The LRRK2 kinase domain deviates from nearly all protein kinases from the consensus DFG…APE activation loop motif [43]. Since the most common LRRK2 mutation G2019S alters the

unusual DYG…APE motif to DYS…APE, this unique region seems critical to the normal regulation of kinase activity, although the serine and threonine residues within the activation loop have not been formally shown as autophosphorylation and possible auto-activation sites. Thus, the high divergence of the LRRK2 kinase domain from other well known protein kinases may provide additional opportunities for specificity in therapeutic approaches but prevents comparisons to homologous kinases for clues to functionality.

Since the first description of LRRK2 kinase activity, additional studies that involve immunoprecipitation of over-expressed LRRK2 protein and assessment of autophosphorylation activity *in vitro* demonstrate that pathogenic PD-linked mutations increase apparent kinase activity [41]. Protein fragments containing the LRRK2 kinase domain isolated from *E. coli* likewise demonstrate auto-phosphorylation activity and kinase activity with MBP as substrate, and the most common LRRK2 mutation, G2019S, imparts a significant increase in kinase activity [44]. Notably, kinase fragments derived from the *E. coli* system are guaranteed devoid of contaminating endogenous serine/threonine and tyrosine kinases. The pathogenic LRRK2 mutation I2020T had no significant effect on activity versus wild-type protein, in contrast with experiments in mammalian systems that demonstrate either increases or decreases in activity [41,45,46]. While the most common LRRK2 mutation G2019S faithfully reproduces enhanced autophosphorylation, other pathogenic LRRK2 mutations fail to reproduce enhanced activity uniformly among different laboratories. G2019S, as opposed to other mutations, may provide an additional autophosphorylation site in the activation loop in addition to enhancing auto-activation that would increase autophosphorylation signal even when a particular assay exceeds linearity. Differences in kinase reaction protocol, contaminating but highly active serine/threonine kinases in kinase reactions, lack of linearity of autophosphorylation assays, lack of a bone fide kinase substrate and sequence differences among different LRRK2 clones and cell lines used for recombinant protein production in different laboratories all likely contribute to variation. Fortunately, the unresolved *in vitro* kinase story does not end with the LRRK2 kinase domain since a second enzymatic domain encoded in the LRRK2 protein, the GTPase domain, provides an additional opportunity to understand the effects of PD-linked LRRK2 mutations on LRRK2 protein function and LRRK2 kinase regulation.

The first descriptions of LRRK2 GTPase activity utilized measurements of GTP-bound LRRK2 protein in cell lysates derived from transiently transfected cells [41]. These estimations revealed that pathogenic PD mutations near the GTPase domain increased the proportion of LRRK2 bound to GTP, while mutations near the kinase domain had no significant effect on the proportion of GTP bound LRRK2. Beyond GTP-bound LRRK2 as a surrogate measure of GTPase activity, more in depth studies reveal that the most common LRRK2 mutations in the LRRK2 GTPase domain, R1441C and R1441G, do not directly enhance GTP binding but convincingly decrease GTP-hydrolysis activity [47–49]. Structural studies demonstrate that the R1441 residue is located at the interface of two GTPase monomer structures and that the mutation likely diminishes GTPase activity from a loss of stabilization of the GTPase dimer [50]. Taken together, the data suggest that the pathogenic PD mutations localized to the GTPase domain prolong the binding to GTP compared to normal LRRK2 protein.

Concurrent with data delineating the effect of PD-associated mutations on GTPase activity, multiple studies report that artificial mutations within the GTPase domain that ablate GTPase activity completely inhibit kinase activity, whereas mutations that ablate kinase activity have no effect on GTPase activity [41,51]. Thus, the LRRK2 protein encodes a self-regulatory module in the GTPase domain that controls kinase output, where mutations that affect GTPase activity necessarily affect kinase activity. This model explains how mutations over 500 amino acid residues away from the kinase domain affect kinase activity, and suggest that pathogenic LRRK2 mutations may influence kinase function either through direct alteration of the kinase domain, alteration of GTPase activity or potentially through altering the interaction of the GTPase and kinase domain. The pathogenic Y1699C mutation localized between the GTPase and kinase domains may alter the structure of LRRK2 protein and influence the contact between the GTPase and kinase domain, although this speculation awaits structural studies with full length recombinant LRRK2 protein.

LRRK2 encodes a protein comprised of elements usually split into two, three or more distinct proteins to form a signal transduction pathway. Descriptions of distant mammalian LRRK relatives in dictyostelium likewise confirm that multiple regulatory domains function in a serial fashion to modify kinase output [52]. In the GbpC protein, a RasGEF domain enhances GTPase activity which in turn is required for kinase activity. Thus, a signal transduction pathway is encoded into a single protein, with opportunities at each step for additional regulation. Perhaps the encoded domains in LRRK2 and related proteins represent such specialized and exact components in controlling kinase output that splitting up the domains into separate proteins would yield insufficient control over kinase output. Regardless of the reason for the unique domains structure, evolution has clearly selected for the arrangement that seemingly positions kinase output as the critical and defining feature of the proteins.

6. LRRK2-linked toxicity and function in cells

Initial efforts to understand the link between the LRRK2 protein and neurotoxicity, namely neurodegeneration that occurs in *LRRK2*-linked PD cases, focused on transient over-expression in cultured cells as a means of delineating the basic aspects of LRRK2 associated dysfunction. The usual caveats and questionable physiological relevance of such approaches apply, nevertheless the most common *LRRK2* mutation G2019S results in increased markers of cellular dysfunction versus wild-type protein expression, suggesting specificity and perhaps a relevant methodology to identify LRRK2-linked cell death pathways [53]. The first demonstration that kinase-activity is required for cellular toxicity, at least *in vitro*, linked together kinase-assay experiments with cell toxicity approaches, and reinforced expectations for the potential therapeutic value of LRRK2 kinase activity [36]. Likewise, expression of either GTPase dead or kinase dead LRRK2 protein is well tolerated by cells, whereas mutant LRRK2 expression correlates with markers of toxicity [41,51].

Towards initial dissection of LRRK2-overexpression linked cell death pathways, transfection of kinase-overactive LRRK2 causes mitochondrial dependent apoptosis blocked by soluble caspase inhibitors or the genetic ablation of Apaf1 [54]. However, the mechanisms that lie in between caspase activation and LRRK2 over-expression remain elusive. The LRRK2 kinase domain contains some sequence homology to the mixed lineage kinase sub-family of MAPKKK proteins, and a unifying feature for MLK proteins is kinase-dependent activation of JNK and activation of c-Jun that leads to apoptosis when over-expressed in transfected cell lines [55]. The MLK proteins have proven control of cell death pathways in neurons since the MLK small molecule inhibitor CEP-1347 provides broad neuroprotection from a variety of neurotoxic insults [56–58]. LRRK2, in contrast to true MLK proteins, does not activate the JNK pathway in a comparable or kinase-dependent manner when transfected into cell lines, suggesting that LRRK2 is not a MAPKKK protein or that the interaction in the MAP-kinase pathway is more complex than usually observed for interacting kinase proteins [41]. Neurons undergoing apoptosis due to LRRK2 over-expression may ultimately have changes in p38 and JNK signaling, although potential perturbation of the MAPK pathway does not automatically assign a role of a MAPKKK to LRRK2 without the identification of specific targets of LRRK2 activity.

LRRK2 kinase output may link over-expression with toxicity, but the relevance of over-expressing an active kinase that may or may not target physiological substrates or native pathways cannot be safely assumed. Indeed, it has not been a requirement to demonstrate endogenous LRRK2 expression in cell systems used to study LRRK2-relevant cell death pathways, and the effects of LRRK2 expression in dopaminergic neurons relevant to PD remain unknown, at least for the time being since this is the current focus of many laboratories. As opposed to the other dominant protein that can cause PD, α-synuclein, LRRK2 expression is very low in the brain compared to reference proteins and is by all accounts difficult to detect by western blot and immunohistochemistry [39]. The importance of LRRK2 kinase activity towards neurodegeneration awaits validation in relevant *in vivo* model systems that more closely mimic endogenous expression.

Initial *in vivo* toxicity experiments in transgenic *Drosophila* that over-express either G2019S LRRK2 or WT LRRK2 protein under the GAL4/UAS system demonstrate LRRK2 linked retinal degeneration and dopaminergic degeneration, with a significantly more severe phenotype in G2019S versus WT LRRK2 over-expressing lines [59]. Unfortunately, the effect of human kinase-dead LRRK2 over-expression was not reported. Imai et al. provide evidence that *Drosophila* LRRK (dLRRK) dysfunction in regulation of protein synthesis leads to degeneration of dopaminergic neurons [60]. Regulation of protein synthesis in their system is proposed to be mediated in part by phosphorylation of the eukaryotic initiation factor 4E binding protein (4E-BP) through dLRRK. However, dLRRK more likely represents LRRK1 function in mammals and genetic variation in LRRK1 does not appear to confer risk for PD [61–63]. Further, loss of dLRRK in different genetic backgrounds of *Drosophila* appears to produce conflicting results with respect to maintenance of dopaminergic neurons [60,64,65]. Deletion of lrk-1 in Nematodes points towards a role of lrk-1 in localizing synaptic vesicle proteins to synaptic terminals, a finding that awaits confirmation in mammalian model organisms to determine whether LRRK1 or LRRK2 or both proteins overlap with lrk-1 function [66].

Given the relatively small tool box from which to analyze LRRK2 protein function, it is no surprise that little is known of the endogenous role of the LRRK2 protein. The strong evolutionary conservation of the protein and broad expression throughout mammalian development in multiple tissues suggests a more ubiquitous function than one confined to the cell types affected in PD. Despite the predicted importance of the gene for normal health and cellular function, the lack of an overt or dramatic phenotype in LRRK2 knockout mice (Saskia Biskup, Valina Dawson, Ted Dawson, unpublished observation) is perhaps no surprise since the knockout of the mouse α-synuclein, parkin and DJ-1 genes, all conserved proteins likely involved in PD, result in only subtle defects. Acute knockdown of LRRK2 via RNA interference is hindered by a lack of knowledge of the endogenous half-life of the LRRK2 protein and by a complete lack of commercially available LRRK2 antibodies that detect rodent LRRK2 protein. Commercial human-specific LRRK2 antibodies are relatively more effective [39], although no described human derived cell line expresses LRRK2 protein detectable by western blot. Possible exceptions like lymphoblastoid cells [21,42] await confirmation in knockdown studies with validated antibodies. Thus, outstanding issues that hinder progress in understanding LRRK2 include effective LRRK2 antibodies and relevant cell systems and corresponding RNAi approaches.

The first study to assign a possible role for over-expressed LRRK2 protein demonstrates that transfection of LRRK2 kinase domain fragments into the intact rodent CNS or full length protein into primary cultures induces changes in neurite length and branching morphology [67]. The reduction of neurite process morphology caused by over-expression of LRRK2 and more-so by expression of G2019S-LRRK2 is regarded as a marker of neurotoxicity and utilized in several studies that estimate LRRK2-mediated cell death [41,51,53]. Two unappealing scenarios might exist that introduce additional complications when interpreting published studies: LRRK2 may endogenously regulate process morphology through mechanisms unrelated to the toxicity associated with LRRK2 expression, therefore LRRK2-toxicity may be over-estimated, or LRRK2 over-expression may non-specifically induce neurite morphology as a downstream consequence of LRRK2 toxicity. Relevant *in vivo* model systems will help resolve the controversy. However, it seems unlikely that the discovery of a clear role for LRRK2 function in neurons will be forthcoming.

7. LRRK2 protein interactors and kinase substrates

If the kinase activity of LRRK2 links LRRK2 function with neurodegeneration in PD, identification and characterization of LRRK2 kinase substrates might provide the shortest route to understanding the role of LRRK2 in disease. *De novo* identification of candidate kinase substrates usually presents a daunting task, ever more difficult with a large kinase protein with little homology to other characterized kinase proteins. Many known kinase substrates interact with kinase proteins in a stable way amenable to visualization through immunoprecipitations or yeast-two hybrid analyses. In transiently transfected cell lines, LRRK2 interacts with several components of the cytoskeleton architecture, including vimentin, a/b-tubulin, and clathrin [68,69]. Immunoprecipitations from brain tissue in mice transgenic for a HA-tagged LRRK2 construct driven by the prion promoter reveal an interaction with HSP-90 [70]. HSP-90 and practically all isoforms have been previously described as common protein interactors with virtually all proteins over-expressed in mammalian cells, as identified in highly purified tandem-affinity purifications [71]. Although it is not known whether LRRK2 might phosphorylate HSP-90 or otherwise functionally contribute to a HSP-90 containing complex, inhibitors to HSP-90 function effectively destabilize transfected and endogenous LRRK2 levels, suggesting that a proportion of LRRK2 interacts with and is stabilized by the inducible HSP-90 protein. Components of the HSP-90 complex in addition to cell cytoskeleton components therefore seem likely targets in the hunt for LRRK2 kinase substrates.

Apart from immunoprecipitations that require stable protein–protein interactions, a novel approach towards identifying kinase substrates dubbed KESTREL (kinase substrate tracking and elucidation), nominated the moesin cytoskeletal protein for further study [46]. LRRK2, in addition to several other serine threonine kinases, phosphorylates moesin at a Thr558 residue, although moesin must be denatured before LRRK2 can phosphorylate this site necessitating further experiments that address whether this interaction occurs in cells. LRRK2 may also affect the phosphorylation of 4E-BP, again targeting sites heavily phosphorylated by other protein kinases [60]. Perhaps the most robust known LRRK2 protein interaction occurs with LRRK2 protein itself, in the form of dimer sized structures and high molecular weight structures [42]. The first observation that LRRK2 might self-interact involved immunoprecipitations between LRRK2 proteins fused to two different epitopes, in this case FLAG and HA tags [45]. Although the immunoprecipitation experiments did not formally demonstrate the existence of a LRRK2 dimeric structure, subsequent studies using native-PAGE and gel filtration experiments demonstrate LRRK2, similar to MLK proteins, forms dimer sized structures [42]. The ability to self-interact might allow dissection of LRRK2 autophosphorylation activity, which in the case of LRRK2, occurs in *cis* [42]. Future studies will determine the effect of LRRK2 quaternary structure on function and kinase activity.

8. Conclusion

From a humble initial description in a large Japanese family to eventually presenting as the most common known cause of PD, the LRRK2 protein provides possibilities for successful disease models,

biochemical characterization of PD, and perhaps most importantly a potentially robust therapeutic target for disease intervention. Before these goals can be fully realized, a number of critical issues as outlined in this review might be considered. The delineation between benign non-synonymous changes and pathogenic mutations is critical for an accurate estimation of the frequency of *LRRK2* mutations in PD patients. Likewise, as the average age of the human population continues to increase and the frequency of age-associated disease like PD rise, genetic counselors will require accurate information particularly if a *LRRK2*-specifc therapy should become available. Studies in North African Arabs screened for the G2019S *LRRK2* mutation demonstrate the challenges of characterization of patient populations where the line between familial and apparently sporadic PD is blurred, a phenomenon that is likely not unique to this population and underscores the difficulty of assigning the role of genetic susceptibility in PD. Thus, the seemingly simple question concerning the true frequency of *LRRK2* mutations in PD even in relatively homogenous populations may not be answered for some time. Perhaps the more pertinent question asks what percentage of PD is caused by LRRK2 activity, and this question will not be fully answered until potent and safe LRRK2 inhibitors become available.

The majority of *LRRK2*-linked PD described in the literature demonstrates clinical and neurochemical changes consistent with the current understanding of idiopathic late-onset PD. Most pathological descriptions of cases with *LRRK2* mutations include SNpc degeneration and Lewy body structures consistent with idiopathic late-onset PD. Notable exceptions include familial cases in the original families that demonstrate both clinical and pathological phenotypes outside of the accepted spectrum for PD. These findings are not unprecedented in monogenic PD; rather, they are expected. For example, the Italian Contursi kindred, where the A53T mutation in the α-*synuclein* gene was first identified, display a broad range of pathologies that can include tau inclusions [72] and diverse clinical manifestations that include a dramatic range of age of disease onset from twenty to eighty-five years of age [73]. For the PD-linked genes described on a pathologic level, namely α-*synuclein*, *parkin*, and *LRRK2*, all can be considered associated with diverse pathology and phenotypes that may not be considered within the current spectrum of idiopathic and sporadic PD. The availability of genetic testing may ultimately broaden the scope of pathologies and clinical manifestations presently associated with PD. However, the unprecedented prevalence of *LRRK2* mutations in PD cases increases opportunities for coincident occurrence of disease unrelated to PD, or additional neurological disease potentially spurred by the development of *LRRK2*-linked PD, both of which might be selected for in the publication process. Thus, when taken together as a whole, the available data suggest that LRRK2 mutations cause a clinical and pathological phenotype that overlaps closely with the heterogeneous PD spectrum.

Although the LRRK2 protein has been linked to PD for only a few years, a world-wide effort to describe LRRK2 and functionally characterize the protein has now provided a solid foundation to move forward. LRRK2 expression in the brain is modest, although the protein is detectable in the neurons vulnerable to degeneration in PD. LRRK2 functions as a kinase and the most common mutation G2019S clearly affects phosphorylation activity. The consequence of LRRK2 kinase activity, whether to enhance phosphorylation of a particular protein substrate or perhaps alter LRRK2 function itself independent of a tertiary kinase substrate awaits further studies and *in vivo* model systems. The sheer size of the LRRK2 open-reading frame introduces a technical challenge for nearly every biochemical application, from the isolation of active and pure recombinant protein to the creation of viral-vector delivery systems. However, studies that utilize fragments of the LRRK2 protein to infer functionality of the full length protein might be interpreted with caution, since all of the protein domains within LRRK2 have not been fully characterized. For example, if the LRRK2 N-terminal repeats specify kinase substrate interactions and this domain is not included in a particular experiment, results that demonstrate kinase-dependent phenotype cannot be ruled out as artifact.

The future might hold the identification of LRRK2 kinase substrates, some of which will be validated *in vivo* while others will remain *in vitro* phenomena, in addition to the development of small molecule LRRK2 inhibitors with variable levels of specificity. The field will rely on model systems that demonstrate a reproducible LRRK2 phenotype to test efficacy of substrate interactions as well as small molecules that might have therapeutic benefit. Since LRRK2 bears strikingly similarity both on a sequence level as well as a functional level to proteins that have been successfully targeted in the treatment of human disease, the prospects are as bright as ever for the development of effective therapeutics to combat PD.

References

[1] J.W. Langston, The Parkinson's complex: parkinsonism is just the tip of the iceberg, Ann. Neurol. 59 (2006) 591–596.
[2] H. Braak, K. Del Tredici, U. Rub, R.A. de Vos, E.N. Jansen Steur, E. Braak, Staging of brain pathology related to sporadic Parkinson's disease, Neurobiol. Aging 24 (2003) 197–211.
[3] M. Funayama, K. Hasegawa, H. Kowa, M. Saito, S. Tsuji, F. Obata, A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2–q13.1, Ann. Neurol. 51 (2002) 296–301.
[4] A. Zimprich, B. Muller-Myhsok, M. Farrer, P. Leitner, M. Sharma, M. Hulihan, P. Lockhart, A. Strongosky, J. Kachergus, D.B. Calne, J. Stoessl, R.J. Uitti, R.F. Pfeiffer, C. Trenkwalder, N. Homann, E. Ott, K. Wenzel, F. Asmus, J. Hardy, Z. Wszolek, T. Gasser, The PARK8 locus in autosomal dominant parkinsonism: confirmation of linkage and further delineation of the disease-containing interval, Am. J. Hum. Genet. 74 (2004) 11–19.
[5] C. Paisan-Ruiz, S. Jain, E.W. Evans, W.P. Gilks, J. Simon, M. van der Brug, A. Lopez de Munain, S. Aparicio, A.M. Gil, N. Khan, J. Johnson, J.R. Martinez, D. Nicholl, I.M. Carrera, A.S. Pena, R. de Silva, A. Lees, J.F. Marti-Masso, J. Perez-Tur, N.W. Wood, A.B. Singleton, Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease, Neuron 44 (2004) 595–600.
[6] L. Bosgraaf, P.J. Van Haastert, Roc, a Ras/GTPase domain in complex proteins, Biochim. Biophys. Acta 1643 (2003) 5–10.
[7] R. Bhandari, N. Srinivasan, M. Mahaboobi, Y. Ghanekar, K. Suguna, S.S. Visweswariah, Functional inactivation of the human guanylyl cyclase C receptor: modeling and mutation of the protein kinase-like domain, Biochemistry 40 (2001) 9196–9206.
[8] A. Di Fonzo, C.F. Rohe, J. Ferreira, H.F. Chien, L. Vacca, F. Stocchi, L. Guedes, E. Fabrizio, M. Manfredi, N. Vanacore, S. Goldwurm, G. Breedveld, C. Sampaio, G. Meco, E. Barbosa, B.A. Oostra, V. Bonifati, A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease, Lancet 365 (2005) 412–415.
[9] W.P. Gilks, P.M. Abou-Sleiman, S. Gandhi, S. Jain, A. Singleton, A.J. Lees, K. Shaw, K.P. Bhatia, V. Bonifati, N.P. Quinn, J. Lynch, D.G. Healy, J.L. Holton, T. Revesz, N.W. Wood, A common LRRK2 mutation in idiopathic Parkinson's disease, Lancet 365 (2005) 415–416.
[10] W.C. Nichols, N. Pankratz, D. Hernandez, C. Paisan-Ruiz, S. Jain, C.A. Halter, V.E. Michaels, T. Reed, A. Rudolph, C.W. Shults, A. Singleton, T. Foroud, Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease, Lancet 365 (2005) 410–412.
[11] M. Funayama, K. Hasegawa, E. Ohta, N. Kawashima, M. Komiyama, H. Kowa, S. Tsuji, F. Obata, An LRRK2 mutation as a cause for the parkinsonism in the original PARK8 family, Ann. Neurol. 57 (2005) 918–921.
[12] S. Goldwurm, A. Di Fonzo, E.J. Simons, C.F. Rohe, M. Zini, M. Canesi, S. Tesei, A. Zecchinelli, A. Antonini, C. Mariani, N. Meucci, G. Sacilotto, F. Sironi, G. Salani, J. Ferreira, H.F. Chien, E. Fabrizio, N. Vanacore, A. Dalla Libera, F. Stocchi, C. Diroma, P. Lamberti, C. Sampaio, G. Meco, E. Barbosa, A.M. Bertoli-Avella, G.J. Breedveld, B.A. Oostra, G. Pezzoli, V. Bonifati, The G6055A (G2019S) mutation in LRRK2 is frequent in both early and late onset Parkinson's disease and originates from a common ancestor, J. Med. Genet. 42 (2005) e65.
[13] D. Gosal, O.A. Ross, J. Wiley, G.B. Irvine, J.A. Johnston, M. Toft, I.F. Mata, J. Kachergus, M. Hulihan, J.P. Taylor, S.J. Lincoln, M.J. Farrer, T. Lynch, J. Mark Gibson, Clinical traits of LRRK2-associated Parkinson's disease in Ireland: a link between familial and idiopathic PD, Parkinsonism Relat. Disord. 11 (2005) 349–352.
[14] J. Kachergus, I.F. Mata, M. Hulihan, J.P. Taylor, S. Lincoln, J. Aasly, J.M. Gibson, O.A. Ross, T. Lynch, J. Wiley, H. Payami, J. Nutt, D.M. Maraganore, K. Czyzewski, M. Styczynska, Z.K. Wszolek, M.J. Farrer, M. Toft, Identification of a novel LRRK2 mutation linked to autosomal dominant parkinsonism: evidence of a common founder across European populations, Am. J. Hum. Genet. 76 (2005) 672–680.
[15] S. Lesage, A.L. Leutenegger, P. Ibanez, S. Janin, E. Lohmann, A. Durr, A. Brice, LRRK2 haplotype analyses in European and North African families with Parkinson disease: a common founder for the G2019S mutation dating from the 13th century, Am. J. Hum. Genet. 77 (2005) 330–332.
[16] C.P. Zabetian, C.M. Hutter, D. Yearout, A.N. Lopez, S.A. Factor, A. Griffith, B.C. Leis, T.D. Bird, J.G. Nutt, D.S. Higgins, J.W. Roberts, D.M. Kay, K.L. Edwards, A. Samii, H.

Payami, LRRK2 G2019S in families with Parkinson disease who originated from Europe and the Middle East: evidence of two distinct founding events beginning two millennia ago, Am. J. Hum. Genet. 79 (2006) 752–758.
[17] D.G. Healy, M. Falchi, S.S. O'Sullivan, V. Bonifati, A. Durr, S. Bressman, A. Brice, J. Aasly, C.P. Zabetian, S. Goldwurm, J.J. Ferreira, E. Tolosa, D.M. Kay, C. Klein, D.R. Williams, C. Marras, A.E. Lang, Z.K. Wszolek, J. Berciano, A.H. Schapira, T. Lynch, K.P. Bhatia, T. Gasser, A.J. Lees, N.W. Wood, Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study, Lancet Neurol. 7 (2008) 583–590.
[18] D.M. Kay, C.P. Zabetian, S.A. Factor, J.G. Nutt, A. Samii, A. Griffith, T.D. Bird, P. Kramer, D.S. Higgins, H. Payami, Parkinson's disease and LRRK2: frequency of a common mutation in U.S. movement disorder clinics, Mov. Disord. 21 (2006) 519–523.
[19] V. Bonifati, LRRK2 low-penetrance mutations (Gly2019Ser) and risk alleles (Gly2385Arg)-linking familial and sporadic Parkinson's disease, Neurochem. Res. 32 (2007) 1700–1708.
[20] J.R. Adams, H. van Netten, M. Schulzer, E. Mak, J. McKenzie, A. Strongosky, V. Sossi, T.J. Ruth, C.S. Lee, M. Farrer, T. Gasser, R.J. Uitti, D.B. Calne, Z.K. Wszolek, A.J. Stoessl, PET in LRRK2 mutations: comparison to sporadic Parkinson's disease and evidence for presymptomatic compensation, Brain 128 (2005) 2777–2785.
[21] H.L. Melrose, C.B. Kent, J.P. Taylor, J.C. Dachsel, K.M. Hinkle, S.J. Lincoln, S.S. Mok, J.G. Culvenor, C.L. Masters, G.M. Tyndall, D.I. Bass, Z. Ahmed, C.A. Andorfer, O.A. Ross, Z.K. Wszolek, A. Delldonne, D.W. Dickson, M.J. Farrer, A comparative analysis of leucine-rich repeat kinase 2 (Lrrk2) expression in mouse brain and Lewy body disease, Neuroscience 147 (2007) 1047–1058.
[22] A.B. West, D.J. Moore, S. Biskup, A. Bugayenko, W.W. Smith, C.A. Ross, V.L. Dawson, T.M. Dawson, Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 16842–16847.
[23] D. Galter, M. Westerlund, A. Carmine, E. Lindqvist, O. Sydow, L. Olson, LRRK2 expression linked to dopamine-innervated areas, Ann. Neurol. 59 (2006) 714–719.
[24] S. Higashi, S. Biskup, A.B. West, D. Trinkaus, V.L. Dawson, R.L. Faull, H.J. Waldvogel, H. Arai, T.M. Dawson, D.J. Moore, P.C. Emson, Localization of Parkinson's disease-associated LRRK2 in normal and pathological human brain, Brain Res. 1155 (2007) 208–219.
[25] S. Higashi, D.J. Moore, R.E. Colebrooke, S. Biskup, V.L. Dawson, H. Arai, T.M. Dawson, P.C. Emson, Expression and localization of Parkinson's disease-associated leucine-rich repeat kinase 2 in the mouse brain, J. Neurochem. 100 (2007) 368–381.
[26] H. Melrose, S. Lincoln, G. Tyndall, D. Dickson, M. Farrer, Anatomical localization of leucine-rich repeat kinase 2 in mouse brain, Neuroscience 139 (2006) 791–794.
[27] J. Simon-Sanchez, V. Herranz-Perez, F. Olucha-Bordonau, J. Perez-Tur, LRRK2 is expressed in areas affected by Parkinson's disease in the adult mouse brain, Eur. J. Neurosci. 23 (2006) 659–666.
[28] J.M. Taymans, C. Van den Haute, V. Baekelandt, Distribution of PINK1 and LRRK2 in rat and mouse brain, J. Neurochem. 98 (2006) 951–961.
[29] J.P. Taylor, M.M. Hulihan, J.M. Kachergus, H.L. Melrose, S.J. Lincoln, K.M. Hinkle, J.T. Stone, O.A. Ross, R. Hauser, J. Aasly, T. Gasser, H. Payami, Z.K. Wszolek, M.J. Farrer, Leucine-rich repeat kinase 1: a paralog of LRRK2 and a candidate gene for Parkinson's disease, Neurogenetics 8 (2007) 95–102.
[30] M. Westerlund, A.C. Belin, A. Anvret, P. Bickford, L. Olson, D. Galter, Developmental regulation of leucine-rich repeat kinase 1 and 2 expression in the brain and other rodent and human organs: implications for Parkinson's disease, Neuroscience 152 (2008) 429–436.
[31] B.S. Han, L. Iacovitti, T. Katano, N. Hattori, W. Seol, K.S. Kim, Expression of the LRRK2 gene in the midbrain dopaminergic neurons of the substantia nigra, Neurosci. Lett. 3 (2008) 190–194.
[32] T. Hatano, S. Kubo, S. Imai, M. Maeda, K. Ishikawa, Y. Mizuno, N. Hattori, Leucine-rich repeat kinase 2 associates with lipid rafts, Hum. Mol. Genet. 16 (2007) 678–690.
[33] S. Biskup, D.J. Moore, F. Celsi, S. Higashi, A.B. West, S.A. Andrabi, K. Kurkinen, S.W. Yu, J.M. Savitt, H.J. Waldvogel, R.L. Faull, P.C. Emson, R. Torp, O.P. Ottersen, T.M. Dawson, V.L. Dawson, Localization of LRRK2 to membranous and vesicular structures in mammalian brain, Ann. Neurol. 60 (2006) 557–569.
[34] G. Perry, X. Zhu, A.K. Babar, S.L. Siedlak, Q. Yang, G. Ito, T. Iwatsubo, M.A. Smith, S.G. Chen, Leucine-rich repeat kinase 2 colocalizes with alpha-synuclein in Parkinson's disease, but not tau-containing deposits in tauopathies, Neurodegener. Dis. 5 (2008) 222–224.
[35] J. Miklossy, T. Arai, J.P. Guo, A. Klegeris, S. Yu, E.G. McGeer, P.L. McGeer, LRRK2 expression in normal and pathologic human brain and in human cell lines, J. Neuropathol. Exp. Neurol. 65 (2006) 953–963.
[36] E. Greggio, S. Jain, A. Kingsbury, R. Bandopadhyay, P. Lewis, A. Kaganovich, M.P. van der Brug, A. Beilina, J. Blackinton, K.J. Thomas, R. Ahmad, D.W. Miller, S. Kesavapany, A. Singleton, A. Lees, R.J. Harvey, K. Harvey, M.R. Cookson, Kinase activity is required for the toxic effects of mutant LRRK2/dardarin, Neurobiol. Dis. 23 (2006) 329–341.
[37] X. Zhu, A. Babar, S.L. Siedlak, Q. Yang, G. Ito, T. Iwatsubo, M.A. Smith, G. Perry, S.G. Chen, LRRK2 in Parkinson's disease and dementia with Lewy bodies, Mol. Neurodegener. 1 (2006) 17.
[38] X. Zhu, S.L. Siedlak, M.A. Smith, G. Perry, S.G. Chen, LRRK2 protein is a component of Lewy bodies, Ann. Neurol. 60 (2006) 617–618 author reply 618–619.
[39] S. Biskup, D.J. Moore, A. Rea, B. Lorenz-Deperieux, C.E. Coombes, V.L. Dawson, T.M. Dawson, A.B. West, Dynamic and redundant regulation of LRRK2 and LRRK1 expression, BMC Neurosci. 8 (2007) 102.
[40] G. Manning, D.B. Whyte, R. Martinez, T. Hunter, S. Sudarsanam, The protein kinase complement of the human genome, Science 298 (2002) 1912–1934.
[41] A.B. West, D.J. Moore, C. Choi, S.A. Andrabi, X. Li, D. Dikeman, S. Biskup, Z. Zhang, K.L. Lim, V.L. Dawson, T.M. Dawson, Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity, Hum. Mol. Genet. 16 (2007) 223–232.
[42] E. Greggio, I. Zambrano, A. Kaganovich, A. Beilina, J.M. Taymans, V. Daniels, P. Lewis, S. Jain, J. Ding, A. Syed, K.J. Thomas, V. Baekelandt, M.R. Cookson, The Parkinson disease-associated leucine-rich repeat kinase 2 (LRRK2) is a dimer that undergoes intramolecular autophosphorylation, J. Biol. Chem. 283 (2008) 16906–16914.
[43] S.K. Hanks, A.M. Quinn, T. Hunter, The protein kinase family: conserved features and deduced phylogeny of the catalytic domains, Science 241 (1988) 42–52.
[44] B. Luzon-Toro, E. Rubio de la Torre, A. Delgado, J. Perez-Tur, S. Hilfiker, Mechanistic insight into the dominant mode of the Parkinson's disease-associated G2019S LRRK2 mutation, Hum. Mol. Genet. 16 (2007) 2031–2039.
[45] C.J. Gloeckner, N. Kinkl, A. Schumacher, R.J. Braun, E. O'Neill, T. Meitinger, W. Kolch, H. Prokisch, M. Ueffing, The Parkinson disease causing LRRK2 mutation I2020T is associated with increased kinase activity, Hum. Mol. Genet. 15 (2006) 223–232.
[46] M. Jaleel, R.J. Nichols, M. Deak, D.G. Campbell, F. Gillardon, A. Knebel, D.R. Alessi, LRRK2 phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity, Biochem. J. 405 (2007) 307–317.
[47] G. Ito, T. Okai, G. Fujino, K. Takeda, H. Ichijo, T. Katada, T. Iwatsubo, GTP binding is essential to the protein kinase activity of LRRK2, a causative gene product for familial Parkinson's disease, Biochemistry 46 (2007) 1380–1388.
[48] P.A. Lewis, E. Greggio, A. Beilina, S. Jain, A. Baker, M.R. Cookson, The R1441C mutation of LRRK2 disrupts GTP hydrolysis, Biochem. Biophys. Res. Commun. 357 (2007) 668–671.
[49] X. Li, Y.C. Tan, S. Poulose, C.W. Olanow, X.Y. Huang, Z. Yue, Leucine-rich repeat kinase 2 (LRRK2)/PARK8 possesses GTPase activity that is altered in familial Parkinson's disease R1441C/G mutants, J. Neurochem. 103 (2007) 238–247.
[50] J. Deng, P.A. Lewis, E. Greggio, E. Sluch, A. Beilina, M.R. Cookson, Structure of the ROC domain from the Parkinson's disease-associated leucine-rich repeat kinase 2 reveals a dimeric GTPase, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 1499–1504.
[51] W.W. Smith, Z. Pei, H. Jiang, V.L. Dawson, T.M. Dawson, C.A. Ross, Kinase activity of mutant LRRK2 mediates neuronal toxicity, Nat. Neurosci. 9 (2006) 1231–1233.
[52] W.N. van Egmond, A. Kortholt, K. Plak, L. Bosgraaf, S. Bosgraaf, I. Keizer-Gunnink, P.J. van Haastert, Intramolecular activation mechanism of the dictyostelium LRRK2-homolog Roco protein GbpC, J. Biol. Chem. 44 (2008) 30412–30420.
[53] W.W. Smith, Z. Pei, H. Jiang, D.J. Moore, Y. Liang, A.B. West, V.L. Dawson, T.M. Dawson, C.A. Ross, Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 18676–18681.
[54] C. Iaccarino, C. Crosio, C. Vitale, G. Sanna, M.T. Carri, P. Barone, Apoptotic mechanisms in mutant LRRK2-mediated cell death, Hum. Mol. Genet. 16 (2007) 1319–1326.
[55] K.A. Gallo, G.L. Johnson, Mixed-lineage kinase control of JNK and p38 MAPK pathways, Nat. Rev. Mol. Cell Biol. 3 (2002) 663–672.
[56] B.L. Apostol, D.A. Simmons, C. Zuccato, K. Illes, J. Pallos, M. Casale, P. Conforti, C. Ramos, M. Roarke, S. Kathuria, E. Cattaneo, J.L. Marsh, L.M. Thompson, CEP-1347 reduces mutant huntingtin-associated neurotoxicity and restores BDNF levels in R6/2 mice, Mol. Cell. Neurosci. 1 (2008) 8–20.
[57] C.G. Besirli, E.M. Johnson Jr., JNK-independent activation of c-Jun during neuronal apoptosis induced by multiple DNA-damaging agents, J. Biol. Chem. 278 (2003) 22357–22366.
[58] J. Lotharius, J. Falsig, J. van Beek, S. Payne, R. Dringen, P. Brundin, M. Leist, Progressive degeneration of human mesencephalic neuron-derived cells triggered by dopamine-dependent oxidative stress is dependent on the mixed-lineage kinase pathway, J. Neurosci. 25 (2005) 6329–6342.
[59] Z. Liu, X. Wang, Y. Yu, X. Li, T. Wang, H. Jiang, Q. Ren, Y. Jiao, A. Sawa, T. Moran, C.A. Ross, C. Montell, W.W. Smith, A *Drosophila* model for LRRK2-linked parkinsonism, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 2693–2698.
[60] Y. Imai, S. Gehrke, H.Q. Wang, R. Takahashi, K. Hasegawa, E. Oota, B. Lu, Phosphorylation of 4E-BP by LRRK2 affects the maintenance of dopaminergic neurons in *Drosophila*, EMBO J. 18 (2008) 2432–2443.
[61] K. Haugarvoll, M. Toft, O.A. Ross, L.R. White, J.O. Aasly, M.J. Farrer, Variants in the LRRK1 gene and susceptibility to Parkinson's disease in Norway, Neurosci. Lett. 416 (2007) 299–301.
[62] I. Marin, The Parkinson disease gene LRRK2: evolutionary and structural insights, Mol. Biol. Evol. 23 (2006) 2423–2433.
[63] I. Marin, Ancient origin of the Parkinson disease gene LRRK2, J. Mol. Evol. 67 (2008) 41–50.
[64] S.B. Lee, W. Kim, S. Lee, J. Chung, Loss of LRRK2/PARK8 induces degeneration of dopaminergic neurons in *Drosophila*, Biochem. Biophys. Res. Commun. 358 (2007) 534–539.
[65] D. Wang, B. Tang, G. Zhao, Q. Pan, K. Xia, R. Bodmer, Z. Zhang, Dispensable role of *Drosophila* ortholog of LRRK2 kinase activity in survival of dopaminergic neurons, Mol. Neurodegener. 3 (2008) 3.
[66] A. Sakaguchi-Nakashima, J.Y. Meir, Y. Jin, K. Matsumoto, N. Hisamoto, LRK-1, a C. elegans PARK8-related kinase, regulates axonal-dendritic polarity of SV proteins, Curr. Biol. 17 (2007) 592–598.
[67] D. MacLeod, J. Dowman, R. Hammond, T. Leete, K. Inoue, A. Abeliovich, The familial Parkinsonism gene LRRK2 regulates neurite process morphology, Neuron 52 (2006) 587–593.

[68] J.C. Dachsel, J.P. Taylor, S.S. Mok, O.A. Ross, K.M. Hinkle, R.M. Bailey, J.H. Hines, J. Szutu, B. Madden, L. Petrucelli, M.J. Farrer, Identification of potential protein interactors of Lrrk2, Parkinsonism Relat. Disord. 13 (2007) 382–385.
[69] P.N. Gandhi, X. Wang, X. Zhu, S.G. Chen, A.L. Wilson-Delfosse, The Roc domain of leucine-rich repeat kinase 2 is sufficient for interaction with microtubules, J. Neurosci. Res. 86 (2008) 1711–1720.
[70] L. Wang, C. Xie, E. Greggio, L. Parisiadou, H. Shim, L. Sun, J. Chandran, X. Lin, C. Lai, W.J. Yang, D.J. Moore, T.M. Dawson, V.L. Dawson, G. Chiosis, M.R. Cookson, H. Cai, The chaperone activity of heat shock protein 90 is critical for maintaining the stability of leucine-rich repeat kinase 2, J. Neurosci. 28 (2008) 3384–3391.
[71] T. Burckstummer, K.L. Bennett, A. Preradovic, G. Schutze, O. Hantschel, G. Superti-Furga, A. Bauch, An efficient tandem affinity purification procedure for interaction proteomics in mammalian cells, Nat. Methods 3 (2006) 1013–1019.
[72] J.E. Duda, B.I. Giasson, M.E. Mabon, D.C. Miller, L.I. Golbe, V.M. Lee, J.Q. Trojanowski, Concurrence of alpha-synuclein and tau brain pathology in the Contursi kindred, Acta Neuropathol. 104 (2002) 7–11.
[73] L.I. Golbe, G. Di Iorio, G. Sanges, A.M. Lazzarini, S. La Sala, V. Bonavita, R.C. Duvoisin, Clinical genetic analysis of Parkinson's disease in the Contursi kindred, Ann. Neurol. 40 (1996) 767–775.
[74] W.C. Nichols, V.E. Elsaesser, N. Pankratz, M.W. Pauciulo, D.K. Marek, C.A. Halter, A. Rudolph, C.W. Shults, T. Foroud, LRRK2 mutation analysis in Parkinson disease families with evidence of linkage to PARK8, Neurology 69 (2007) 1737–1744.
[75] G. Xiromerisiou, G.M. Hadjigeorgiou, V. Gourbali, J. Johnson, I. Papakonstantinou, A. Papadimitriou, A.B. Singleton, Screening for SNCA and LRRK2 mutations in Greek sporadic and autosomal dominant Parkinson's disease: identification of two novel LRRK2 variants, Eur. J. Neurol. 14 (2007) 7–11.
[76] C. Paisan-Ruiz, P. Nath, N. Washecka, J.R. Gibbs, A.B. Singleton, Comprehensive analysis of LRRK2 in publicly available Parkinson's disease cases and neurologically normal controls, Hum. Mutat. 29 (2008) 485–490.
[77] D. Berg, K. Schweitzer, P. Leitner, A. Zimprich, P. Lichtner, P. Belcredi, T. Brussel, C. Schulte, S. Maass, T. Nagele, Type and frequency of mutations in the LRRK2 gene in familial and sporadic Parkinson's disease*, Brain 128 (2005) 3000–3011.
[78] D. Haubenberger, S. Bonelli, C. Hotzy, P. Leitner, P. Lichtner, D. Samal, R. Katzenschlager, A. Djamshidian, T. Brucke, M. Steffelbauer, C. Bancher, J. Grossmann, G. Ransmayr, T.M. Strom, T. Meitinger, T. Gasser, E. Auff, A. Zimprich, A novel LRRK2 mutation in an Austrian cohort of patients with Parkinson's disease, Mov. Disord. 22 (2007) 1640–1643.
[79] L. Skipper, H. Shen, E. Chua, C. Bonnard, P. Kolatkar, L.C. Tan, R.D. Jamora, K. Puvan, K.Y. Puong, Y. Zhao, R. Pavanni, M.C. Wong, Y. Yuen, M. Farrer, J.J. Liu, E.K. Tan, Analysis of LRRK2 functional domains in nondominant Parkinson disease, Neurology 65 (2005) 1319–1321.
[80] A.M. Schlitter, D. Woitalla, T. Mueller, J.T. Epplen, G. Dekomien, The LRRK2 gene in Parkinson's disease: mutation screening in patients from Germany, J. Neurol. Neurosurg. Psychiatry 77 (2006) 891–892.
[81] A.S. Chen-Plotkin, W. Yuan, C. Anderson, E. McCarty Wood, H.I. Hurtig, C.M. Clark, B.L. Miller, V.M. Lee, J.Q. Trojanowski, M. Grossman, V.M. Van Deerlin, Corticobasal syndrome and primary progressive aphasia as manifestations of LRRK2 gene mutations, Neurology 70 (2008) 521–527.
[82] K. Nuytemans, R. Rademakers, J. Theuns, P. Pals, S. Engelborghs, B. Pickut, T. de Pooter, K. Peeters, M. Mattheijssens, M. Van den Broeck, P. Cras, P.P. De Deyn, C. van Broeckhoven, Founder mutation p.R1441C in the leucine-rich repeat kinase 2 gene in Belgian Parkinson's disease patients, Eur. J. Hum. Genet. 16 (2008) 471–479.
[83] C. Paisan-Ruiz, A.E. Lang, T. Kawarai, C. Sato, S. Salehi-Rad, G.K. Fisman, T. Al-Khairallah, P. St George-Hyslop, A. Singleton, E. Rogaeva, LRRK2 gene in Parkinson disease: mutation analysis and case control association study, Neurology 65 (2005) 696–700.
[84] C.P. Zabetian, A. Samii, A.D. Mosley, J.W. Roberts, B.C. Leis, D. Yearout, W.H. Raskind, A. Griffith, A clinic-based study of the LRRK2 gene in Parkinson disease yields new mutations, Neurology 65 (2005) 741–744.
[85] I.F. Mata, J.M. Kachergus, J.P. Taylor, S. Lincoln, J. Aasly, T. Lynch, M.M. Hulihan, S.A. Cobb, R.M. Wu, C.S. Lu, C. Lahoz, Z.K. Wszolek, M.J. Farrer, Lrrk2 pathogenic substitutions in Parkinson's disease, Neurogenetics 6 (2005) 171–177.
[86] S.N. Pchelina, A.F. Yakimovskii, A.K. Emelyanov, O.N. Ivanova, A.L. Schwarzman, A. B. Singleton, Screening for LRRK2 mutations in patients with Parkinson's disease in Russia: identification of a novel LRRK2 variant, Eur. J. Neurol. 15 (2008) 692–696.
[87] N.L. Khan, S. Jain, J.M. Lynch, N. Pavese, P. Abou-Sleiman, J.L. Holton, D.G. Healy, W.P. Gilks, M.G. Sweeney, M. Ganguly, V. Gibbons, S. Gandhi, J. Vaughan, L.H. Eunson, R. Katzenschlager, J. Gayton, G. Lennox, T. Revesz, D. Nicholl, K.P. Bhatia, N. Quinn, D. Brooks, A.J. Lees, M.B. Davis, P. Piccini, A.B. Singleton, N.W. Wood, Mutations in the gene LRRK2 encoding dardarin (PARK8) cause familial Parkinson's disease: clinical, pathological, olfactory and functional imaging and genetic data, Brain 128 (2005) 2786–2796.
[88] S. Lesage, S. Janin, E. Lohmann, A.L. Leutenegger, L. Leclere, F. Viallet, P. Pollak, F. Durif, S. Thobois, V. Layet, M. Vidailhet, Y. Agid, A. Durr, A. Brice, A.M. Bonnet, M. Borg, E. Broussolle, P. Damier, A. Destee, M. Martinez, C. Penet, O. Rasco, F. Tison, C. Tranchan, M. Verin, LRRK2 exon 41 mutations in sporadic Parkinson disease in Europeans, Arch. Neurol. 64 (2007) 425–430.
[89] J. Clarimon, J. Pagonabarraga, C. Paisan-Ruiz, A. Campolongo, B. Pascual-Sedano, J.F. Marti-Masso, A.B. Singleton, J. Kulisevsky, Tremor dominant parkinsonism: clinical description and LRRK2 mutation screening, Mov. Disord. 23 (2008) 518–523.
[90] L.J. Ozelius, G. Senthil, R. Saunders-Pullman, E. Ohmann, A. Deligtisch, M. Tagliati, A.L. Hunt, C. Klein, B. Henick, S.M. Hailpern, R.B. Lipton, J. Soto-Valencia, N. Risch, S.B. Bressman, LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews, N. Engl. J. Med. 354 (2006) 424–425.
[91] J.C. Moller, I. Rissling, V. Mylius, C. Hoft, K.M. Eggert, W.H. Oertel, The prevalence of the G2019S and R1441C/G/H mutations in LRRK2 in German patients with Parkinson's disease, Eur. J. Neurol. 15 (2008) 743–745.
[92] J.M. Bras, R.J. Guerreiro, M.H. Ribeiro, C. Januario, A. Morgadinho, C.R. Oliveira, L. Cunha, J. Hardy, A. Singleton, G2019S dardarin substitution is a common cause of Parkinson's disease in a Portuguese cohort, Mov. Disord. 20 (2005) 1653–1655.
[93] A. Di Fonzo, C. Tassorelli, M. De Mari, H.F. Chien, J. Ferreira, C.F. Rohe, G. Riboldazzi, A. Antonini, G. Albani, A. Mauro, R. Marconi, G. Abbruzzese, L. Lopiano, E. Fincati, M. Guidi, P. Marini, F. Stocchi, M. Onofrj, V. Toni, M. Tinazzi, G. Fabbrini, P. Lamberti, N. Vanacore, G. Meco, P. Leitner, R.J. Uitti, Z.K. Wszolek, T. Gasser, E.J. Simons, G.J. Breedveld, S. Goldwurm, G. Pezzoli, C. Sampaio, E. Barbosa, E. Martignoni, B.A. Oostra, V. Bonifati, Comprehensive analysis of the LRRK2 gene in sixty families with Parkinson's disease, Eur. J. Hum. Genet. 14 (2006) 322–331.
[94] I.F. Mata, O.A. Ross, J. Kachergus, C. Huerta, R. Ribacoba, G. Moris, M. Blazquez, L.M. Guisasola, C. Salvador, C. Martinez, M. Farrer, V. Alvarez, LRRK2 mutations are a common cause of Parkinson's disease in Spain, Eur. J. Neurol. 13 (2006) 391–394.
[95] S. Lesage, L. Leclere, E. Lohmann, M. Borg, M. Ruberg, A. Durr, A. Brice, Frequency of the LRRK2 G2019S mutation in siblings with Parkinson's disease, Neurodegener. Dis. 4 (2007) 195–198.
[96] G.D. Mellick, G.A. Siebert, M. Funayama, D.D. Buchanan, Y. Li, Y. Imamichi, H. Yoshino, P.A. Silburn, N. Hattori, Screening PARK genes for mutations in early-onset Parkinson's disease patients from Queensland, Australia, Parkinsonism Relat. Disord. XX (2008) 1–5.
[97] M.M. Pimentel, K.C. Moura, C.B. Abdalla, J.S. Pereira, A.L. de Rosso, D.H. Nicaretta, M. Campos Jr., R.M. de Almeida, J.M. dos Santos, I.C. Bastos, M.F. Mendes, H. Maultasch, F.H. Costa, A.L. Werneck, C.B. Santos-Reboucas, A study of LRRK2 mutations and Parkinson's disease in Brazil, Neurosci. Lett. 433 (2008) 17–21.
[98] J.O. Aasly, M. Toft, I. Fernandez-Mata, J. Kachergus, M. Hulihan, L.R. White, M. Farrer, Clinical features of LRRK2-associated Parkinson's disease in central Norway, Ann. Neurol. 57 (2005) 762–765.
[99] D.A. Grimes, L. Racacho, F. Han, M. Panisset, D.E. Bulman, LRRK2 screening in a Canadian Parkinson's disease cohort, Can. J. Neurol. Sci. 34 (2007) 336–338.
[100] N. Dupre, J.B. Riviere, R.H. Myers, P. Provencher, E. Pourcher, F. Emond, G.A. Rouleau, LRRK2 is not a significant cause of Parkinson's disease in French-Canadians, Can. J. Neurol. Sci. 34 (2007) 333–335.
[101] K. Kalinderi, L. Fidani, S. Bostantjopoulou, Z. Katsarou, A. Kotsis, The G2019S LRRK2 mutation is uncommon amongst Greek patients with sporadic Parkinson's disease, Eur. J. Neurol. 14 (2007) 1088–1090.
[102] M. Bialecka, S. Hui, G. Klodowska-Duda, G. Opala, E.K. Tan, M. Drozdzik, Analysis of LRRK 2 G 2019 S and I 2020 T mutations in Parkinson's disease, Neurosci. Lett. 390 (2005) 1–3.
[103] E.K. Tan, Y. Zhao, L. Skipper, M.G. Tan, A. Di Fonzo, L. Sun, S. Fook-Chong, S. Tang, E. Chua, Y. Yuen, L. Tan, R. Pavanni, M.C. Wong, P. Kolatkar, C.S. Lu, V. Bonifati, J.J. Liu, The LRRK2 Gly2385Arg variant is associated with Parkinson's disease: genetic and functional evidence, Hum. Genet. 120 (2007) 857–863.
[104] O.A. Ross, Y.R. Wu, M.C. Lee, M. Funayama, M.L. Chen, A.I. Soto, I.F. Mata, G.J. Lee-Chen, C.M. Chen, M. Tang, Y. Zhao, N. Hattori, M.J. Farrer, E.K. Tan, R.M. Wu, Analysis of Lrrk2 R1628P as a risk factor for Parkinson's disease, Ann. Neurol. 64 (2008) 88–92.
[105] C. Gaig, M. Ezquerra, M.J. Marti, F. Valldeoriola, E. Munoz, A. Llado, M.J. Rey, A. Cardozo, J.L. Molinuevo, E. Tolosa, Screening for the LRRK2 G2019S and codon-1441 mutations in a pathological series of parkinsonian syndromes and frontotemporal lobar degeneration, J. Neurol. Sci. 270 (2008) 94–98.
[106] O.A. Ross, M. Toft, A.J. Whittle, J.L. Johnson, S. Papapetropoulos, D.C. Mash, I. Litvan, M.F. Gordon, Z.K. Wszolek, M.J. Farrer, D.W. Dickson, Lrrk2 and Lewy body disease, Ann. Neurol. 59 (2006) 388–393.
[107] B.I. Giasson, J.P. Covy, N.M. Bonini, H.I. Hurtig, M.J. Farrer, J.Q. Trojanowski, V.M. Van Deerlin, Biochemical and pathological characterization of Lrrk2, Ann. Neurol. 59 (2006) 315–322.
[108] A. Rajput, D.W. Dickson, C.A. Robinson, O.A. Ross, J.C. Dachsel, S.J. Lincoln, S.A. Cobb, M.L. Rajput, M.J. Farrer, Parkinsonism, Lrrk2 G2019S, and tau neuropathology, Neurology 67 (2006) 1506–1508.
[109] Z.K. Wszolek, R.F. Pfeiffer, Y. Tsuboi, R.J. Uitti, R.D. McComb, A.J. Stoessl, A.J. Strongosky, A. Zimprich, B. Muller-Myhsok, M.J. Farrer, T. Gasser, D.B. Calne, D.W. Dickson, Autosomal dominant parkinsonism associated with variable synuclein and tau pathology, Neurology 62 (2004) 1619–1622.
[110] M.T. Giordana, C. D'Agostino, G. Albani, A. Mauro, A. Di Fonzo, A. Antonini, V. Bonifati, Neuropathology of Parkinson's disease associated with the LRRK2 Ile1371Val mutation, Mov. Disord. 22 (2007) 275–278.